Test Procedure for

SOIL ORGANIC CONTENT USING UV-VIS METHOD

TxDOT Designation: Tex-148-E

Effective Date: October 2020

1. SCOPE

1.1 This method determines the Soil Organic Content (SOC) based on the amount of humic acid present in the soil sample by using Ultraviolet-Visible Spectroscopy (UV-Vis).

1.2 UV-Vis uses a beam of light with a wavelength of 300 nanometers to pass through a sample in a cuvette. The amount of light absorbance of the sample is measured and used to determine the SOC.

2. DEFINITIONS

2.1 *Blank*–Solution that consists of reagents without soil sample.

2.2 *Constant Weight*-Soils are oven-dried at a temperature of 104 ± 5°F, such that they will not lose more than 0.1% moisture after 4 hr. of drying. Verify constant weight by measuring the moisture content from weighing a sample before and after consecutive 4-hr. drying periods.

2.3 *Cuvette*–A small, transparent tube of square cross section sealed at one end, made of fused quartz and designed to hold sample solution for spectroscopic analysis.

2.4 *Filtrate Solution*–Soil and reagents that has passed through a filter.

2.5 *Reagents*–a chemical compound of a known purity.

2.6 *Ultraviolet-Visible Spectroscopy (UV-Vis)*—The process of measuring the soil organic content by the use of UV-Vis light absorption properties from soil extracts.

3. APPARATUS

3.1 *Balance,* Class A in accordance with Tex-901-K.

3.2 *Bottles*, 1 L, glass or polyethylene for storage of 1N hydrochloric acid and sodium pyrophosphate solutions (minimum of two required).

3.3 *Centrifuge tubes,* 50 mL polypropylene (minimum of four required).

3.4 *Crusher.*

3.5 *Cuvettes,* 10 mm path length, capable of measuring at a wavelength of 300nm (minimum of seven required).

3.6 *Distilled or deionized water.*

3.7 *Funnel.*

3.8 *Graduated cylinder* (glass or plastic), 10 mL.

3.9 *Latex gloves.*

3.10 *Lint-free wipes*.

3.11 *Mortar and pestle.*

3.12 *Oven* (optional).

3.13 *Sample splitter.*

3.14 *Sieves,* U.S. Standard No. 4 and No. 40.

3.15 *Stopwatch.*

3.16 *Syringes,* Luer-lok 10 mL.

3.17 *Syringe filter,* 25 mm filter with 0.45 µm polypropylene membrane.

3.18 *UV-Vis spectrometer*, capable of measuring at a minimum wavelength of 300 nm.

3.19 *Volumetric flasks,* 500 mL and 1000 mL.

3.20 *Wash bottles*, 500 mL (minimum of three required).

4. REPORTING AND DOCUMENTATION

4.1 Report all data and information pertinent to this soil using the SiteManager form 'tx148.xlsm'.

Note 1-This form is available from the Materials & Tests Division/Soils & Aggregates Section. It is also available online at the following link http://www.txdot.gov/inside-txdot/forms-publications/consultants-contractors/forms/site-manager.html.

5. MATERIALS

5.1 *Hydrochloric Acid solution* – 1.0N (Certified).

5.2 *Humic acid powder* – laboratory grade, CAS number 1415-93-6, used to prepare standards with organic contents of 0.5, 1.0, and 1.5%.

5.3 *Sodium Pyrophosphate Decahydrate* – Crystalline/Certified ACS reagent grade.

5.4 *Sodium Hydroxide* – ACS reagent grade pellets.

6. PROCEDURES

6.1 *This test procedure does not claim to address the safety concerns associated with its use. It is the responsibility of the user of this test procedure to establish the appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations before use.*

6.2 ***Preparing Test Sample***

6.2.1 Obtain a representative soil sample in accordance with Tex-100-E. Sample a minimum of 5 lbs. of soil at approximately in the middle of the proposed treatment layer.

6.2.2 Dry the sample in an oven at 104±9°F to constant weight, as defined in Section 2.2. Allow the sample to cool to room temperature. Alternatively, air-dry the sample to constant weight.

6.2.3 Crush and grind the entire dried sample to pass the No. 4 sieve.

6.2.4 Split the sample to obtain approximately 300 g.

6.2.5 Pulverize the 300 g sample to pass the No. 40 sieve.

6.2.6 Use a spatula and stir the pulverized sample. Weigh three individual soil test samples at 0.10±0.01g per sample, place. each sample into individual centrifuge tubes and label them appropriately.

6.3 Ensure all lab equipment used when preparing reagents, standards, blank and test samples are clean and free of any residue before use.

6.4 ***Preparing Reagents***

6.4.1 *Prepare Hydrochloric Acid solution.*

6.4.1.1 Pour approximately 500 mL of Hydrochloric Acid solution from Section 5.1 into an empty wash bottle using a funnel and label it appropriately.

6.4.2 *Prepare Sodium Pyrophosphate solution.*

6.4.2.1 Pour 500 mL of distilled or deionized water into an empty 1,000 mL volumetric flask using a funnel.

6.4.2.2 Weigh 44.6±0.1 g of Sodium Pyrophosphate Decahydrate from Section 5.3 and place it into the flask.

6.4.2.3 Weigh 10.0±0.1 g of Sodium Hydroxide from Section 5.4 and place it into the flask.

6.4.2.4 Add additional distilled or deionized water into the flask until it reaches the 1,000 mL mark. Stir the solution until the chemicals have dissolved.

6.4.2.5 Pour approximately 500 mL of Sodium Pyrophosphate solution into an empty wash bottle using a funnel and label it appropriately.

6.4.2.6 Pour remaining solutions into individual polyethylene bottles and label them appropriately. Store the bottles for future testing.

Note 2-Chemicals should not be stored near heat sources such as laboratory ovens or in direct sunlight.

6.5 ***Preparing Standard Samples***

6.5.1 Label three individual 500 mL volumetric flasks as 0.5%, 1.0% and 1.5% respectively.

6.5.2 Pour 100 mL of Hydrochloric Acid solution from Section 6.4.1 into each flask.

6.5.3 Add 400 mL of Sodium Pyrophosphate solution to each flask.

6.5.4 Prepare the 0.5% standard sample. Weigh 0.01±0.001 g of humic acid powder from Section 5.2 and carefully place it into the appropriate flask not to lose any powder.

6.5.5 Prepare the 1.0% standard sample. Weigh 0.02±0.001 g of humic acid powder and carefully place into the appropriate flask not to lose any powder.

6.5.6 Prepare the 1.5% standard sample. Weigh 0.03±0.001 g of humic acid powder and carefully place into the appropriate flask not to lose any powder.

6.5.7 Stir the solution in each flask until the chemicals have dissolved.

6.5.8 Label three syringes from Section 3.16 as 0.5%, 1.0% and 1.5% respectively.

6.5.9 Use the appropriate syringe and draw a minimum of 5 mL from each standard sample.

6.5.10 Attach a new syringe filter from Section 3.17 to each syringe. Use a new syringe filter for each syringe.

6.5.11 Fill one cuvette for each standard sample.

6.5.12 Pour remaining solutions into individual polyethylene bottles and label them appropriately. Store the bottles for future testing.

Note 3-Chemicals should not be stored near heat sources such as laboratory ovens or in direct sunlight.

6.6 ***Preparing Blank and Test Samples***

6.6.1 Pour 5 mL of hydrochloric acid solution from Section 6.4.1 into a graduated cylinder.

6.6.2 Pour the 5 mL into a centrifuge tube and label it 'Blank.'

6.6.3 Repeat 6.6.1 and pour 5 mL into each test sample prepared in 6.2.

6.6.4 Vigorously shake each centrifuge tube of soil and Hydrochloric Acid solution by hand for 10 sec. at 1-min. intervals, five times.

6.6.5 Temporarily store each centrifuge tube in a vertical position after shaking.

6.6.6 Add 20 mL of Sodium Pyrophosphate solution prepared from Section 6.4.2 into a graduated cylinder.

6.6.7 Pour the 20 mL into the centrifuge tube labeled 'Blank.'

6.6.8 Repeat 6.6.6 and pour 20 mL into each centrifuge tube containing the test samples prepared in 6.2.

6.6.9 Vigorously shake each centrifuge tube of soil and Sodium Pyrophosphate solution by hand for 10 sec. at 1-min. intervals, five times.

6.6.10 Store each centrifuge tube in a vertical position after shaking for a minimum of 15 min. to allow the soil samples to settle and the air bubbles to dissipate.

6.6.11 Label four additional clean syringes from Section 3.16 as 'Blank' and for each test sample prepared in 6.2.

6.6.12 Use the appropriate syringe and draw a minimum of 5 mL from each centrifuge tube in Section 6.6.10.

6.6.13 Attach a syringe filter from Section 3.17 to each syringe. Use a new syringe filter for each syringe.

6.6.14 Fill one cuvette for the blank and each test sample.

6.7 ***Measuring Soil Organic Content***

6.7.1 Prepare the UV-Vis spectrometer for testing at a wavelength of 300 nanometers according to the manufacturer's guidelines and recommendations.

6.7.2 Use latex gloves and wipe the sides of each cuvette from Section 7.5.14 using a lint-free wipe to remove fingerprints and any other residue.

Note 4–Fingerprints and residue will obstruct the light beam from the spectrometer that passes through the cuvette and will produce inaccurate test results.

6.7.3 Visually inspect the cuvettes for bubbles and particulates. When bubbles and particulates are present, do not test the cuvette. Prepare a new cuvette sample.

6.7.4 Place a cuvette into the UV-Vis spectrometer in the order of blank; 0.5%, 1.0%, and 1.5% standard samples; and test samples.

6.7.5 Determine the absorbance value of each cuvette and enter the results into the SiteManager template in Section 4.1. The template will graph the absorbance values from each cuvette, determine an R-square value, and calculate the soil organic content of each test sample.

6.7.6 When the R-square value of the graph is less than 0.9500, prepare and test additional blank and standard soil samples to improve the best-fit line to achieve the minimum R-square value.

Note 5- Refer to the manufacturer's guidelines and recommendations for the UV-Vis spectrometer when an R-square value of 0.9500 or greater cannot be achieved.

7. CALCULATIONS

7.1 Calculate the soil organic content of each test sample:

$$SOC = \frac{y - b}{m}$$

Where:

$SOC = Soil\ Organic\ Content, \%$

$y = absorbance$

$m = slope\ of\ best\ fit\ line$

$b = y\ intercept$

7.2 Record the soil organic content to the nearest 0.1%.

7.3 Calculate the average soil organic content using the results of the three test samples.

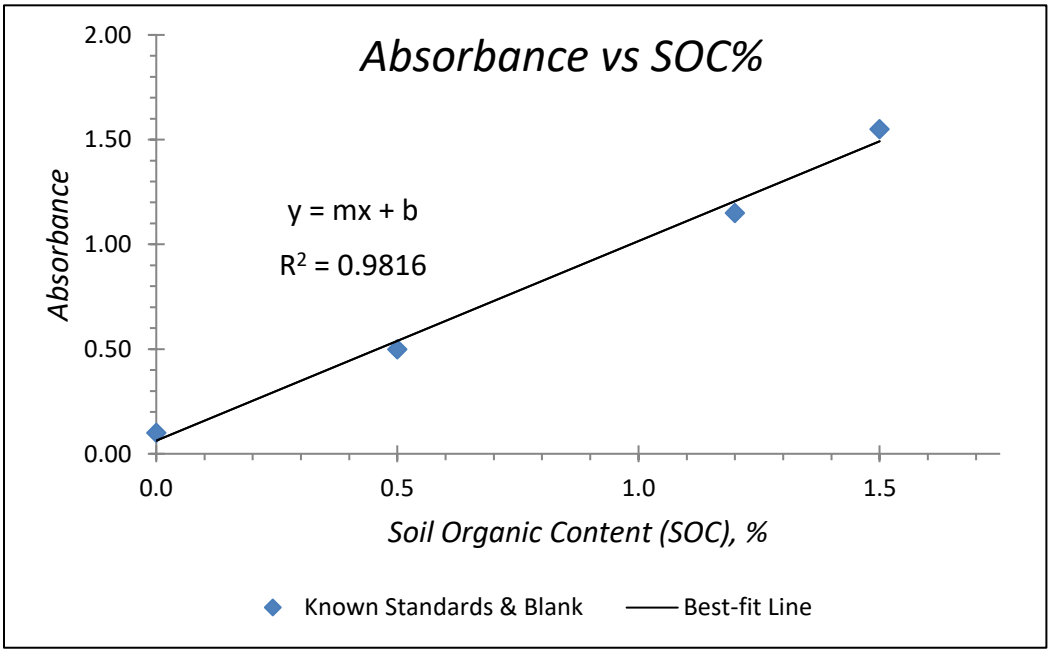

Figure 1 - Example of Soil Organic Content (SOC) vs. Absorbance

8. TEST REPORT

8.1 Report the soil organic content of the soil samples as a percent to the nearest 0.1.